PULSE ELECTRIC FIELD EXPOSURE EFFECT ON MORPHOLOGICAL PROPERTIES OF HELA CELLS

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For my beloved father, Mr. Adon bin Mohd Pendik; my wife,
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ABSTRACT

This thesis is concerned with the investigation of pulsed electric field (PEF) towards biological cells. Biological cells selected in this study are HeLa (cervical cancer) cells. There are two parts of the study, which was involving modeling methods and experimental setup. Modeling method used involves analytical (MATLAB) and numerical (CST®EMS) methods. Both of these methods are to prove the existence of the effect on transmembrane potential changes when subjected exposed to PEF strength. This result can be seen clearly when both method showed the existence of changes effects on transmembrane potential. Therefore, this study continues by identifying an appropriate experimental setup. Experimental setup involves four important parts, the first part is the source of square wave PEF (ECM®830) that can generate until 3kV field strength. Followed by modified EC magnetic chamber with incubator system that has been used in order to exposed HeLa cells to PEF. At the same time this system is coupled with Nikon inverted microscope (Ti-series) for subsequent visualization techniques, image and video. In the early stage, experimental setup was tested by monitoring the proliferation rate of HeLa cells within 0 to 48 hours. Then HeLa cells were tested to look at the swelling effect via PEF exposure. After that, we continued to identify the optimum PEF parameters for reversible condition on HeLa cell. As a result HeLa cells gives a good response at 2.7kV field strength, 30μs pulse length with single pulse. Further study showed that two or more adjacent HeLa cells merge together due to increased cell membrane permeability (electrofusion). This discovery triggered an idea to look at the PEF effect on wound healing process. An artificial wound site were investigated with and without PEF exposure. The finding shows PEF exposed wound area took 3 hours to completely heal while the untreated area took 10 hours. This prove a novel technique (electrical based novel treatment) which could be an alternative to drug usage for wound healing process. Overall, the findings achieved in this study could lead us onto a drug free wound healing method.

ABSTRAK

Tesis ini menjurus kepada penyiasatan medan elektrik denyut (PEF) terhadap sel biologi. Sel biologi yang dipilih dalam kajian ini adalah sel HeLa (kanser servik). Kajian ini terbahagi kepada dua bentuk iaitu kaedah permodelan dan eksperimen. Kaedah permodelan yang terlibat adalah kaedah beranalisis (MATLAB) dan berangka (CST®EMS). Kedua-dua kaedah ini adalah untuk membuktikan wujudnya perubahan upaya terhadap sel transmembran apabila didedahkan kepada kekuatan PEF. Keputusannya, secara jelas menunjukkan bahawa wujudnya perubahan upaya terhadap sel transmembran. Sehubungan dengan itu, kajian ini diteruskan mengenalpasti persediaan eksperimen yang sesuai. Eksperimen ini melibatkan empat bahagian yang utama, bahagian pertama adalah sumber gelombang segiempat medan elektrik denyut (ECM®830) yang boleh menghasilkan kekuatan medan sehingga 3kV. Diikuti dengan pengubahsuaian kebuk magnet EC dengan sistem pengeraman bagi kegunaan pendedahan PEF terhadap sel HeLa. Pada masa yang sama sistem tersebut digandingkan dengan mikroskop (Ti-series) tersongsang Nikon untuk teknik gambaran imej dan video. Pada peringkat awal, persediaan eksperimen ini diuji dengan pengawasan kadar pertumbuhan sel HeLa antara 0 hingga 48 jam. Kemudian sel HeLa diuji untuk melihat kesan pengembangan apabila PEF dikenakan. Seterusnya mengenalpasti parameter optimum PEF untuk keadaan boleh balik sel HeLa pada kekuatan medan 2.7kV, 30μs panjang denyut tunggal. Kemudian, kajian diteruskan dengan melihat kenaikan kebolehtelapan sel membran (elektro-pelakuran) terhadap PEF menyebabkan dua atau lebih sel HeLa yang bersebelahan bergabung menjadikannya bentuk sel yang besar. Penemuan ini menjadi pemicu kepada idea untuk melihat kesan PEF terhadap proses penyembuhan luka. Sehubungan dengan itu, kajian terhadap luka buatan dibangunkan mnggunakan sel HeLa di mana, sampel sel tersebut dibahagikan kepada dua bahagian iaitu didedahkan dan tidak didedahkan dengan PEF. Keputusannya memperlihatkan hanya 3 jam diambil untuk penyembuhan luka jika dikenakan PEF berbanding 10 jam dalam keadaan biasa. Kesimpulannya, teknik novel (rawatan berasaskan elektrik) boleh menjadi alternatif kepada penggunaan dadah untuk proses penyembuhan luka seterusnya pencetus kepada kaedah yang bebas dadah dalam penyembuhan luka.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

CST® EMS	Computer Simulation Technology®Electromagnetic Studio
ECM®830	Square Wave Pulse Generator
EP	Electroporation
FIT	Finite Integral Technique
PEF	Pulse Electric Field
TA	Threshold Area
HELA	Cervical Cancer Cell

LIST OF PUBLICATIONS, RESEARCH GRANT AWARD AND RESEARCH COMPETITION

The followings are the list of publications relevant to this thesis.

Book Chapter:

1. **Adon, M Nazib**, M Noh Dalimin, N. M. Kassim, and M.M A.Jamil. 2011. Microdosimetry Modeling Technique for Spherical Cell." In *5th Kuala Lumpur International Conference on Biomedical Engineering 2011: BIOMED 2011, 20-23 June 2011, Kuala Lumpur, Malaysia*: Springer. 447.

Conference Proceedings:

2. **Adon, M. N**., M. Noh Dalimin, M. M. A. Jamil, and N. M. Kassim. 2012. "Development of high voltage pulse inducement method for biological cell." In *Biomedical Engineering (ICoBE), 2012 International Conference on*. 501-503.

3. **Adon, M. N**., M. Noh Dalimin, M. M. A. Jamil, N. M. Kassim, and S. Hamdan. 2012. "Study of effect of microsecond pulsed electric fields on threshold area of HeLa cells." In *Biomedical Engineering and Sciences (IECBES), 2012 IEEE EMBS Conference on*. 484-486.

4. **Adon, M. N.**, M. Noh Dalimin, M. M. A. Jamil, N. M. Kassim, and S. Hamdan. 2013. "Electrofusion effect of induced transmembrane potential under a live - Cell microscopy system." In *Biomedical Engineering International Conference (BMEiCON), 2013 6th*. 1-3.

5. Zaltum, Milad, A Mohamed, **Adon, M.N.**, M. M. A. Jamil. 2013. "Electroporation effect on growth of HeLa cells." In *Biomedical Engineering International Conference (BMEiCON), 2013 6th*: IEEE. 1-4.

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CHAPTER 1

INTRODUCTION

Human cell biology system is too complex to understand. Most scientists have been doing a lot of research intensively to understand the effects of stimulus that occurs between the human cell biological system against external factors, including the effects of electric fields applied in different intensities and durations. In this chapter we will emphasize of discovery the new phenomenon electric field impact on human cell biology performed.

1.1. Background of Research

The earliest written record on biological cell effects of electric fields have been reported over the past fourth decades. Neumann firstly reported in 1972 permeability changes induced by pulsed electric field in cell membrane (Neuman. and Rosenheck. 1972). Zimmermann explained the permeability changes as a pore formation of membranes due to its electrical breakdown, such as electroporation (Zimmerman., Pilwat. *et al.* 1974). In other words, electroporation is a technique in which electric pulses are used to create transient pores in the cell membrane used for the delivery of biologically active molecules into cells (Smith, Neu *et al.* 2004).

Most studies relating electroporation behavior are involving in-vitro techniques which engage various processes of culturing human cells. Among the cells used in this process include melanoma cells (Petrishia and Sasikala 2014), J3T (brain tumors) cells (Neal Ii, Rossmeisl Jr *et al.* 2014), Chinese hamster ovary (CHO-K1) cells (Thompson, Roth *et al.* 2014), and HeLa (cervical cancer) cells (Zhang, Xiong *et al.* 2013). Therefore there are a variety of cells that can be used and categorized it as different cell shape, structure and content. Thus cell selection is very significant in the process of electroporation which every type of cell has a different consequence towards electric field intensities and duration.

Since the structures of cells are too complex, various modeling methods are introduced to represent the cells structure with simplified model have been used to studying the cells, such as circuit model (Roy and Barman 2014), parallel plate models (Hsiao, Choi *et al.* 2013), and the layered model (Mesin 2013). In essence, it is very useful to examine transmembrane potential, pore formation, reversible and irreversible electroporation process in greater depth and detail .

However, to prove the validity of the model, real time experimental setup must be develop to verify the existence of electric field effects on cells. Previous study showed there are two areas of study involving the experimental analysis of electroporation process namely bioscience and bioengineering . Most biotechnology application research involves electroporation to allowing chemicals, drugs or DNA to be introduced into the cell which is relevant for the purposes of medical applications and genetic studies (Lindstrom, Brewer *et al.* 2014). In contrast, bioengineering research concentrate on transient aqueous pores form in lipid bilayers, that is fundamental mechanism of large electric fields may alter physiological and morphological on cell (Thompson, Payne *et al.* 2011). Therefore, the requirement of high voltage intensity with multiple pulses are indispensable in realizing each experiment carried out effectively.

Recently, the unique pulsed electric field effects against biological cell, has opened a new gateway to tumor treatment and become a research focus in the area of bioelectromagnetics (Ferreira, Saga *et al.* 2013; Gehl, Linnert *et al.* 2013; Mali, Jarm *et al.* 2013). There are numerous experimental research show that pulsed electric field with different parameters can cause different bioelectric effects. Weaver et al. found that in response to microsecond pulsed electric field (typical parameters: 1

kV/cm, 100μs), many reversible aqueous channels, which are often called pores (radius ≈20-110 nm), appear at the cell membrane, while there is no obvious effect on the transmembrane organelles. This physical procedure is termed electroporation, which can make cell membrane more permeable to drug molecule. The following technique has been successfully applied to tumor treatment (Weaver 2000; Weaver 2003).

However, most of the electroporation experiments using a cuvette to hold samples with a variety of electrode gap sizes. Three electrode gap sizes are available, 1mm for bacteria and yeast, 2mm for all cell types and 4mm for mammalian cells. The cuvette are molded with embedded polished aluminum electrodes, and gamma irradiated for guaranteed sterility. Nonetheless, this cuvette system cannot be integrated with real time visualization using high resolution microscopy.

Therefore, the experimental setup with environment controlled system must be identified to make all observation during pulse electric field induced can be recorded in real time visualization with high resolution microscopy system. As a result it could be concluded that pulsed electric field with different duration and intensity can cause various biomedical effects such as morphological changes on cells, which suggests a various types of external pulsed electric field and biological cell. Since then there have been a number of studies on the mechanism of electroporation and its applications to gene transfection (Dower, Miller *et al.* 1988), cell fusion (Zimmermann, Friedrich *et al.* 2000) and medical treatments (Dev, Rabussay *et al.* 2000).

1.1.1. Introduction to Electroporation

The developmental of micro and molecular biology, chemical and biological techniques have been developed to transfer the selected material through the cellular membrane (Ausubel, Brent *et al.* 2002). The capabilities of implemented transmembrane transport of materials is crucial to many areas of research. Most studies involve the transport of macromolecules such as DNA, RNA, antibodies, chemical drugs, metabolites, molecular probes and multiple vesicles.

Research related to electroporation (EP) has attracted more scholarly among cell biologists and biophysicists, is that high-voltage electric pulses can generate fusion cells. Giant cells viability was first obtained by Neumann et al. (Neumann, Gerisch *et al.* 1980) with a simple electro-pulsing of a suspension of cells. Later, it was proposed to use the phenomenon of dielectrophoresis (Pohl 1978), to obtain close contact between cells (Scheurich, Zimmermann *et al.* 1981). Dielectrophoresis is the movement of a relatively nonconducting particles or charged (cells) in a non-uniform AC electric fields (Pohl 1978). If several particles are present, the appropriate particle size, particle density, electric field magnitude and frequency can cause the cells to aggregate in long chains (pearl chain) in an alternating electric field depending on their effective polarisability (Zimmermann 1982).

Neumann et al. (Neumann, Schaefer-Ridder *et al.* 1982) have reported a method of transfection of foreign genes into eukaryotic cells by electroporation method. Transfection involves opening transient pores in the plasma membrane of cells, to permit the taking of genetic material or proteins such as antibodies. It was also reported that the transfected genes expressed in the host cells (Neumann, Schaefer-Ridder *et al.* 1982). Following the discovery, electroporation has become accepted as an effective technique for the introduction of foreign DNA into cells of any origin (Potter 1988; Neumann 1989).

Further techniques have been developed in various fields including biochemistry, genetics, medicine, pharmacology, immunology, microbiology and toxicology. Utilities in vivo electroporation for the entry of molecules has been demonstrated by the increasing number of new applications have been developed each year (Mir 2001).

Experiments in 1988 on human skin fibroblast showed that highly efficient transient chloramphenicol acetyltransferase expression was shown after transfection with plasmid (Fountain, Lockwood *et al.* 1988). The ability to easily transfect these cells with exogenous DNA may have important applications in the study of human genetic diseases and cancer. Further research has shown that the electroporation of the skin could be used to enhance transdermal drug delivery (Prausnitz, Bose *et al.* 1993; Prausnitz, Pliquett *et al.* 1994) .

In 1993 it was reported that upon application of electric fields pulses on a suspension of cells in the presence of a selected membrane protein, implantation of the protein in the cell plasma membrane was possible (Nicolau, Mouneimne *et al.* 1993). This phenomenon is called electroinsertion. Later, electroporation of excitable membranes was observed (O'Neill and Tung 1991). Electrically induced membrane breakdown of isolated cardiomyocytes cells was reported the morphological evidence for the presence of RhoA protein (Wang, Tsai *et al.* 1997).

A method of electroporation has been applied in vivo to introduce anticancer drugs to tumorous tissue (electrochemotheraphy) in order to obtain therapeutic effects by using short, intense electric pulses that surpass the capacitance of the cell membrane (Gothelf, Mir *et al.* 2003). The main factors that play a crucial role in obtaining high responses of the treatment are the drug used in the treatment and the appropriate electric pulses delivered to the tumor.

Electroporation has been focuses on the selective sterilization of fermented foods by intense pulsed electric field. Since intense electric field can make pores penetrating a membrane of a cell, the principle of the sterilization by intense electric field is irreversible electroporation that releases the contents through the pores of the cell membrane of microorganisms, it is simpler and more efficient than rival chemical and biological processes (Manabe, Nakagawa *et al.* 2011; Saito, Minamitani *et al.* 2013; Manabe, Maetani *et al.* 2014).

However, the mechanism of electroporation is still not fully understood and there are aspects of the process that has yet to be reviewed to see its effectiveness. Which is related to the parameters of electric pulses optimal for specific cell to the desired application (Hibino, Itoh *et al.* 1993; Jaroszeski, Heller *et al.* 2000; Satkauskas and Saulis 2004). The scope of this thesis is to address some of these problems in an attempt to increase understanding and efficiency through optimization of parameters for electroporation.

1.2. Problem Formulation

However, the inducement process of external pulse electric field towards cells is still not completely understood. Therefore, there is need for supplementing experimental knowledge with theoretical models (Valič, Golzio *et al.* 2003; Krassowska and Filev 2007; Chengxiang, Chenguo *et al.* 2011; Moen, Roth *et al.* 2013). This is due to the small size and thickness of cells and their thin membranes resulting in great difficulty for experimental investigation and applicable of numerical technique.

In this thesis, analytical and quasistatic approximation techniques have been used to investigate the interaction of pulsed electric field with biological cells. The outcome of these assessments will guide the experimental evaluations of pulsed electric field influences on biological materials at cellular level. More specifically, the studies presented in this thesis will clarify; (i) the field intensity and potential difference built on cell membrane; (ii) development of experimental setup for real time study of morphological features on cell membrane under pulsed electric field exposure combined with high resolution microscopy imaging features.

1.3. Aims and Objectives

The primary aim of this study is to examine the phenomenon of biological effect on electric field pulses towards HeLa cell. To enable this, the thesis is presented in two main parts:

Part I: In which the work will firstly investigate the association between the effects of the electric field strength that produces towards spherical cell model for transmembrane potential, where the analytical and numerical models developed for spherical cell to prove this theory. This will then lead towards development of controlled electroporation technique for HeLa cells, to improve this process using live imaging techniques in real time (Chapter 3, 4 and 5).

Part II: In which the newly established electroporation technique will be investigated to examine optimization of pulsed electrical field (PEF) and the number of pulse towards morphological changes on HeLa cell. This will then lead towards phenomena of electrofusion and thus, discovery of wound healing process assisted by electric field excitation (Chapter 6 and 7).

1.4. Scope of Research

In order to achieve the objective of this research, following scope of work have been identified which comprises of:

1. Modeling and simulation of single and multi layer for spherical cell shape based on quasistatic approximation approach by analytical and numerical method.
2. Preparing a complete HeLa cell culture protocol on 25cm^2 flask in medical instrumentation laboratory.
3. Develop a real time visualization setup for pulse electric field effect exposure on HeLa cells.
4. Preparing a complete HeLa cell culture protocol on CMB and EC magnetic chamber for control environment and real time visualization.
5. Characterize an optimization of high voltage pulse electric filed exposure for electroporation process.
6. Real time morphological observation of multicellular behavior during PEF exposure for qualitative analysis.

1.5. Overview of Thesis

This thesis has been organized into nine chapter as follows:

Chapter 1 Introduction: A brief review of the topic and a general introduction will be focused in this chapter. The fascinating aspects on relationship between biological cells and pulse electric field studies will further investigated. According to previous

REFERENCES

Abidor, I., V. Arakelyan, et al. (1979). Electric breakdown of bilayer lipid membranes: I. The main experimental facts and their qualitative discussion. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* 104: 37-52.

Abidor, I. G., V. B. Arakelyan, et al. (1979). Electric breakdown of bilayer lipid membranes: I. The main experimental facts and their qualitative discussion. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* 104(0): 37-52.

Adon, M. N., M. N. Dalimin, et al. (2012). Development of high voltage pulse inducement method for biological cell. *Biomedical Engineering (ICoBE), 2012 International Conference on*.

Agbaba, J., I. Ivanev-Tumbas, et al. (2004). Formation of by-products in the course of intermediate ozonation of groundwater pretreated with ozone and polyaluminium chloride. *Water Science & Technology* 49(4): 63-68.

André, F. M., J. Gehl, et al. (2008). Efficiency of high-and low-voltage pulse combinations for gene electrotransfer in muscle, liver, tumor, and skin. *Human Gene Therapy* 19(11): 1261-1272.

Andreason, G. L. and G. A. Evans (1989). Optimization of electroporation for transfection of mammalian cell lines. *Analytical Biochemistry* 180(2): 269-275.

Appert, N. (1810). *L'art de conserver pendant plusieurs années toutes les substances animales et végétales*, chez Patris.

Argyris, J., K. Buck, et al. (1968). Some new elements for the matrix displacement method, DTIC Document.

Ausubel, F. M., R. Brent, et al. (2002). *Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology*, Wiley New York.

Ayuni, E. L., A. Gazdhar, et al. (2010). In vivo electroporation mediated gene delivery to the beating heart. *PLoS One* 5(12): e14467.

Balanis, C. A. (1989). *Advanced engineering electromagnetics*, Wiley New York.

Benz, R., F. Beckers, et al. (1979). Reversible electrical breakdown of lipid bilayer membranes: A charge-pulse relaxation study. *The Journal of Membrane Biology* 48(2): 181-204.

Bertacchini, C., P. M. Margotti, et al. (2007). Design of an irreversible electroporation system for clinical use. *Technology in cancer research & treatment* 6(4): 313-320.

Bhobe, A. U., C. Holloway, et al. (2001). Meander delay line challenge problem: a comparison using FDTD, FEM and MoM. *Electromagnetic Compatibility, 2001. EMC. 2001 IEEE International Symposium on*, IEEE.

Blaese, R. M., K. W. Culver, et al. (1995). T lymphocyte-directed gene therapy for ADA− SCID: initial trial results after 4 years. *Science* 270(5235): 475-480.

Blair-Parks, K., B. C. Weston, et al. (2002). High-level gene transfer to the cornea using electroporation. *The journal of gene medicine* 4(1): 92-100.

Borges, R. M., J. H. Horne, et al. (2013). A detailed description of an economical setup for electroporation of chick embryos in ovo. *Brazilian Journal of Medical and Biological Research* 46(9): 752-757.

Bureau, M., J. Gehl, et al. (2000). Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1474(3): 353-359.

Casey, P. J. (1995). Protein lipidation in cell signaling. *Science (New York, N.Y.)* 268(5208): 221-225.

Castro, A. J., G. V. Barbosa Canovas, et al. (1993). Microbial inactivation of foods by pulsed electric fields. *Journal of Food Processing and Preservation* 17(1): 47-73.

Cemazar, M., M. Golzio, et al. (2011). Hyaluronidase and collagenase increase the transfection efficiency of gene electrotransfer in various murine tumors. *Human Gene Therapy* 23(1): 128-137.

Cemazar, M., T. Jarm, et al. (2010). Cancer electrogene therapy with interleukin-12. *Current Gene Therapy* 10(4): 300-311.

Chang, D. and T. S. Reese (1990). Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. *Biophysical Journal* 58(1): 1-12.

Chang, D. C., B. M. Chassy, et al. (1992). *Guide to electroporation and electrofusion*, Academic Press.

Chang, D. C. and T. S. Reese (1990). Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. *Biophysical Journal* 58(1): 1-12.

Chengxiang, L., Y. Chenguo, et al. (2011). Dependence on electric field intensities of cell biological effects induced by microsecond pulsed electric fields. *Dielectrics and Electrical Insulation, IEEE Transactions on* 18(6): 2083-2088.

Chernomordik, L., S. Sukharev, et al. (1983). Breakdown of lipid bilayer membranes in an electric field. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 736(2): 203-213.

Chernomordik, L. V., S. I. Sukharev, et al. (1983). Breakdown of lipid bilayer membranes in an electric field. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 736(2): 203-213.

Chiarella, P., V. M Fazio, et al. (2010). Application of electroporation in DNA vaccination protocols. *Current Gene Therapy* 10(4): 281-286.

Cole, K. S. (1968). *Membranes, ions, and impulses: a chapter of classical biophysics*, Univ of California Press.

Coustets, M., V. Ganeva, et al. (2014). Millisecond duration pulses for flow-through electro-induced protein extraction from E. coli and associated eradication. *Bioelectrochemistry*.

Curtis, H. and N. S. Barnes. (1983). *Biology*. New York, Worth Publishing.

Daud, A. I., R. C. DeConti, et al. (2008). Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. *Journal of Clinical Oncology* 26(36): 5896-5903.

De Vry, J., P. Martinez-Martinez, et al. (2010). In vivo electroporation of the central nervous system: A non-viral approach for targeted gene delivery. *Progress in Neurobiology* 92(3): 227-244.

Dean, D., D. Machado-Aranda, et al. (2003). Electroporation as a method for high-level nonviral gene transfer to the lung. *Gene Therapy* 10(18): 1608-1615.

DeBruin, K. A. and W. Krassowska (1999). Modeling electroporation in a single cell. I. Effects of field strength and rest potential. *Biophysical Journal* 77(3): 1213-1224.

Deng, J., K. H. Schoenbach, et al. (2003). The effects of intense submicrosecond electrical pulses on cells. *Biophysical Journal* 84(4): 2709-2714.

Dev, S. B., D. P. Rabussay, et al. (2000). Medical applications of electroporation. *Plasma Science, IEEE Transactions on* 28(1): 206-223.

Diederich, A., G. Bähr, et al. (1998). Influence of surface charges on the rupture of black lipid membranes. *Physical Review E* 58(4): 4883-4889.

Dimitrov, D. (1995). Electroporation and electrofusion of membranes. *Handbook of Biological Physics* 1: 851-901.

Dower, W. J., J. F. Miller, et al. (1988). High efficiency transformation of E.coli by high voltage electroporation. *Nucleic Acids Research* 16(13): 6127-6145.

Drago, G. and S. Ridella (1982). Evaluation of electrical fields inside a biological structure. *The British journal of cancer. Supplement* 5: 215.

Edd, J. F., L. Horowitz, et al. (2006). In vivo results of a new focal tissue ablation technique: irreversible electroporation. *Biomedical Engineering, IEEE Transactions on* 53(7): 1409-1415.

Ellappan, P. and R. Sundararajan (2005). A simulation study of the electrical model of a biological cell. *Journal of Electrostatics* 63(3): 297-307.

Escoffre, J.-M., T. Portet, et al. (2009). What is (still not) known of the mechanism by which electroporation mediates gene transfer and expression in cells and tissues. *Molecular Biotechnology* 41(3): 286-295.

Ferreira, D. M., Y. Y. Saga, et al. (2013). Chitosan Nanoparticles for Melanoma Cancer Treatment by Photodynamic Therapy and Electrochemotherapy Using Aminolevulinic Acid Derivatives. *Current medicinal chemistry*.

Fountain, J. W., W. K. Lockwood, et al. (1988). Transfection of primary human skin fibroblasts by electroporation. *Gene* 68(1): 167-172.

Framme, C., G. Schuele, et al. (2004). Influence of pulse duration and pulse number in selective RPE laser treatment. *Lasers in Surgery and Medicine* 34(3): 206-215.

Fricke, H. (1924). A mathematical treatment of the electric conductivity and capacity of disperse systems I. The electric conductivity of a suspension of homogeneous spheroids. *Physical Review* 24(5): 575.

Furse, C., D. A. Christensen, et al. (2009). *Basic introduction to bioelectromagnetics*, CRC press.

Gabriel, B. and J. Teissié (1997). Direct observation in the millisecond time range of fluorescent molecule asymmetrical interaction with the electropermeabilized cell membrane. *Biophysical Journal* 73(5): 2630-2637.

Gabriel, B. and J. Teissié (1998). Mammalian cell electropermeabilization as revealed by millisecond imaging of fluorescence changes of ethidium bromide in interaction with the membrane. *Bioelectrochemistry and Bioenergetics* 47(1): 113-118.

Garcia, P. A., R. E. Neal, et al. (2011). An experimental investigation of temperature changes during electroporation. *General Assembly and Scientific Symposium, 2011 XXXth URSI*.

Gaynor, P. and P. Bodger (1995). Electrofusion processes: theoretical evaluation of high electric field effects on cellular transmembrane potentials. *IEE Proceedings-Science, Measurement and Technology* 142(2): 176-182.

Gaynor, P., D. Wells, et al. (2005). Couplet alignment and improved electrofusion by dielectrophoresis for a zona-free high-throughput cloned embryo production system. *Medical and Biological Engineering and Computing* 43(1): 150-154.

Gehl, J. (2003). Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. *Acta Physiologica Scandinavica* 177(4): 437-447.

Gehl, J., M. Linnert, et al. (2013). *Current treatments for patients with multiple brain metastases focusing on electrochemotherapy*.

Ghosh, P. M., C. R. Keese, et al. (1993). Monitoring electropermeabilization in the plasma membrane of adherent mammalian cells. *Biophysical Journal* 64(5): 1602-1609.

Gimsa, J. and D. Wachner (1998). A unified resistor-capacitor model for impedance, dielectrophoresis, electrorotation, and induced transmembrane potential. *Biophysical Journal* 75(2): 1107-1116.

Glaser, R. W., S. L. Leikin, et al. (1988). Reversible electrical breakdown of lipid bilayers: formation and evolution of pores. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 940(2): 275-287.

Gothelf, A., L. M. Mir, et al. (2003). Electrochemotherapy: Results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treatment Reviews* 29(5): 371-387.

Gowrishankar, T. R. and J. C. Weaver (2003). An approach to electrical modeling of single and multiple cells. *Proceedings of the National Academy of Sciences* 100(6): 3203-3208.

Griese, T., S. Kakorin, et al. (2002). Conductometric and electrooptic relaxation spectrometry of lipid vesicle electroporation at high fields. *Physical Chemistry Chemical Physics* 4(7): 1217-1227.

Guo, S., A. Donate, et al. (2011). Electro-gene transfer to skin using a noninvasive multielectrode array. *Journal of Controlled Release* 151(3): 256-262.

Gurtovenko, A. A., J. Anwar, et al. (2010). Defect-Mediated Trafficking across Cell Membranes: Insights from in Silico Modeling. *Chemical Reviews* 110(10): 6077-6103.

Gusbeth, C., W. Frey, et al. (2009). Critical comparison between the pulsed electric field and thermal decontamination methods of hospital wastewater. *Acta Physica Polonica-Series A General Physics* 115(6): 1092.

Gusbeth, C., W. Frey, et al. (2009). Pulsed electric field treatment for bacteria reduction and its impact on hospital wastewater. *Chemosphere* 75(2): 228-233.

Haas, C. and D. Aturaliye (1999). Semi-quantitative characterization of electroporation-assisted disinfection processes for inactivation of Giardia and Cryptosporidium. *Journal of applied microbiology* 86(6): 899-905.

Haberl, S., D. Miklavčič, et al. (2010). Effect of Mg ions on efficiency of gene electrotransfer and on cell electropermeabilization. *Bioelectrochemistry* 79(2): 265-271.

Hamdi, F. S., O. Français, et al. (2014). How medium osmolarity influences dielectrophoretically assisted on-chip electrofusion. *Bioelectrochemistry* 100(0): 27-35.

Harrington, R. F. (1967). Matrix methods for field problems. *Proceedings of the IEEE* 55(2): 136-149.

Hibino, M., H. Itoh, et al. (1993). Time courses of cell electroporation as revealed by submicrosecond imaging of transmembrane potential. *Biophysical Journal* 64(6): 1789-1800.

Hibino, M., M. Shigemori, et al. (1991). Membrane conductance of an electroporated cell analyzed by submicrosecond imaging of transmembrane potential. *Biophysical Journal* 59(1): 209-220.

Hjouj, M., D. Last, et al. (2012). MRI study on reversible and irreversible electroporation induced blood brain barrier disruption. *PLoS One* 7(8): e42817.

Ho, S. and G. Mittal (1996). Electroporation of cell membranes: a review. *Critical reviews in biotechnology* 16(4): 349-362.

Hofmann, G. A. and G. A. Evans (1986). Electronic genetic-physical and biological aspects of cellular electromanipulation. *Engineering in Medicine and Biology Magazine, IEEE* 5(4): 6-25.

Hovis, K. R., K. Padmanabhan, et al. (2010). A simple method of in vitro electroporation allows visualization, recording, and calcium imaging of local neuronal circuits. *Journal of Neuroscience Methods* 191(1): 1-10.

Hsiao, C. T., J. K. Choi, et al. (2013). Modelling single-and tandem-bubble dynamics between two parallel plates for biomedical applications. *Journal of Fluid Mechanics* 716: 137-170.

Huang, Y., N. S. Sekhon, et al. (2003). Instantaneous, quantitative single-cell viability assessment by electrical evaluation of cell membrane integrity with microfabricated devices. *Sensors and Actuators A: Physical* 105(1): 31-39.

Huber, R., V. Treyer, et al. (2005). Exposure to pulse-modulated radio frequency electromagnetic fields affects regional cerebral blood flow. *European Journal of Neuroscience* 21(4): 1000-1006.

Huo, R., Q. Ma, et al. (2010). Noninvasive Electromagnetic Fields on Keratinocyte Growth and Migration. *The Journal of surgical research* 162(2): 299-307.

Ireland, J. C., P. Klostermann, et al. (1993). Inactivation of Escherichia coli by titanium dioxide photocatalytic oxidation. *Applied and environmental microbiology* 59(5): 1668-1670.

Jaroszeski, M. J., R. Gilbert, et al. (2000). Electrically mediated reporter gene transfer into normal rat liver tissue. *Electrochemotherapy, Electrogenetherapy, and Transdermal Drug Delivery*, Springer**:** 333-338.

Jaroszeski, M. J., R. Heller, et al. (2000). Electrochemotherapy, electrogenetherapy, and transdermal drug delivery. *Humana, Totowa, NJ.*

Joshi, R. and Q. Hu (2010). Analysis of cell membrane permeabilization mechanics and pore shape due to ultrashort electrical pulsing. *Medical and Biological Engineering and Computing* 48(9): 837-844.

Joshi, R. P., A. Mishra, et al. (2010). Model study of time-dependent muscle response to pulsed electrical stimulation. *Bioelectromagnetics* 31(5): 361-370.

Kandušer, M., D. Miklavčič, et al. (2009). Mechanisms involved in gene electrotransfer using high-and low-voltage pulses—an in vitro study. *Bioelectrochemistry* 74(2): 265-271.

Khan, O. G. M. and A. H. El-Hag (2011). Biological cell electroporation using nanosecond electrical pulses. *Biomedical Engineering (MECBME), 2011 1st Middle East Conference on*.

Kim, J. A., K. Cho, et al. (2008). A novel electroporation method using a capillary and wire-type electrode. *Biosensors and Bioelectronics* 23(9): 1353-1360.

Kinosita, K., M. Hibino, et al. (1992). Events of membrane electroporation visualized on a time scale from microsecond to seconds. *Guide to electroporation and electrofusion*: 29-46.

Kinosita, K. and T. Y. Tsong (1977). Formation and resealing of pores of controlled sizes in human erythrocyte membrane.

Knisley, S. B. and A. O. Grant (1995). Asymmetrical electrically induced injury of rabbit ventricular myocytes. *Journal of Molecular and Cellular Cardiology* 27(5): 1111-1122.

Knorr, D. and A. Angersbach (1998). Impact of high-intensity electric field pulses on plant membrane permeabilization. *Trends in Food Science & Technology* 9(5): 185-191.

Kotnik, T., P. Kramar, et al. (2012). Cell membrane electroporation- Part 1: The phenomenon. *Electrical Insulation Magazine, IEEE* 28(5): 14-23.

Kotnik, T. and D. Miklavčič (2006). Theoretical Evaluation of Voltage Inducement on Internal Membranes of Biological Cells Exposed to Electric Fields. *Biophysical Journal* 90(2): 480-491.

Kotnik, T., D. Miklavčič, et al. (1998). Time course of transmembrane voltage induced by time-varying electric fields—a method for theoretical analysis and its application. *Bioelectrochemistry and Bioenergetics* 45(1): 3-16.

Kotnik, T., L. M. Mir, et al. (2001). Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses: Part I. Increased efficiency of permeabilization. *Bioelectrochemistry* 54(1): 83-90.

Kotnik, T., G. Pucihar, et al. (2003). Role of pulse shape in cell membrane electropermeabilization. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1614(2): 193-200.

Krassowska, W. and P. D. Filev (2007). Modeling Electroporation in a Single Cell. *Biophysical Journal* 92(2): 404-417.

Krietenstein, B., R. Schuhmann, et al. (1998). The perfect boundary approximation technique facing the big challenge of high precision field computation. *Proceedings of the XIX International Linear Accelerator Conference (LINAC 98), Chicago, USA*.

Kulbacka, J., M. Nowak, et al. (2011). The influence of electroporation on in vitro photodynamic therapy of human breast carcinoma cells. *Folia Biologica (Czech Republic)* 57(3): 112-118.

Lawaczeck, R. (1988). Defect Structures in Membranes: Routes for the Permeation of Small Molecules. *Berichte der Bunsengesellschaft für physikalische Chemie* 92(9): 961-963.

Leontiadou, H., A. E. Mark, et al. (2004). Molecular Dynamics Simulations of Hydrophilic Pores in Lipid Bilayers. *Biophysical Journal* 86(4): 2156-2164.

Li, S. (2004). Electroporation gene therapy: New developments in vivo and in vitro. *Current Gene Therapy* 4(3): 309-316.

Li, X., P. M. Vlahovska, et al. (2013). Continuum-and particle-based modeling of shapes and dynamics of red blood cells in health and disease. *Soft Matter* 9(1): 28-37.

Lindstrom, Z. K., S. J. Brewer, et al. (2014). Injection of propidium iodide into HeLa cells using a silicon nanoinjection lance array. *Journal of Nanotechnology in Engineering and Medicine* 5(2).

Lipowsky, R. (1995). *Structure and dynamics of membranes*, North Holland.

Litster, J. D. (1975). Stability of lipid bilayers and red blood cell membranes. *Physics Letters A* 53(3): 193-194.

Liu, L.-M. and S. F. Cleary (1995). Absorbed energy distribution from radiofrequency electromagnetic radiation in a mammalian cell model: Effect of membrane-bound water. *Bioelectromagnetics* 16(3): 160-171.

Liu, L. M. and S. F. Cleary (1995). Absorbed energy distribution from radiofrequency electromagnetic radiation in a mammalian cell model: Effect of membrane-bound water. *Bioelectromagnetics* 16(3): 160-171.

Mali, B., T. Jarm, et al. (2013). The Influence of Tumor Size on Effectiveness of Electrochemotherapy. *World Congress on Medical Physics and Biomedical Engineering May 26-31, 2012, Beijing, China*. M. Long, Springer Berlin Heidelberg. 39**:** 1600-1603.

Manabe, Y., M. Maetani, et al. (2014). Influences of pulsed electric fields on the gene expression of pathogenic bacteria. *IEEJ Transactions on Fundamentals and Materials* 134(6): 390-396+393.

Manabe, Y., R. Nakagawa, et al. (2011). Influences of pulsed electric fields on the gene expression of pathogenic bacteria. *Digest of Technical Papers-IEEE International Pulsed Power Conference*.

Marshall, E. (1999). Gene therapy death prompts review of adenovirus vector. *Science* 286(5448): 2244-2245.

Masters, J. R. W. (2000). Human cancer cell lines: fact and fantasy. *Nat Rev Mol Cell Biol* 1(3): 233-236.

Melikov, K. C., V. A. Frolov, et al. (2001). Voltage-Induced Nonconductive Pre-Pores and Metastable Single Pores in Unmodified Planar Lipid Bilayer. *Biophysical Journal* 80(4): 1829-1836.

Mesin, L. (2013). Volume conductor models in surface electromyography: Computational techniques. *Computers in Biology and Medicine* 43(7): 942-952.

Méthot, S., V. Moulin, et al. (2001). Morphological changes of human skin cells exposed to a DC electric field in vitro using a new exposure system. *The Canadian Journal of Chemical Engineering* 79(4): 668-677.

Miklavcic, D. and T. Kotnik (2004). Electroporation for electrochemotherapy and gene therapy. *Bioelectromagnetic medicine*: 637-656.

Milne, W. E. and W. Milne (1953). *Numerical solution of differential equations*, Wiley New York.

Mir, L. M. (2001). Therapeutic perspectives of in vivo cell electropermeabilization. *Bioelectrochemistry* 53(1): 1-10.

Mir, L. M., S. Orlowski, et al. (1991). Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses. *European Journal of Cancer and Clinical Oncology* 27(1): 68-72.

Mir, L. M., S. Orlowski, et al. (1995). Biomedical applications of electric pulses with special emphasis on antitumor electrochemotherapy. *Bioelectrochemistry and Bioenergetics* 38(1): 203-207.

Mitsutake, K., A. Satoh, et al. (2010). Study of effect of pulsing sequence of nanosecond pulsed electric fields on viability of HeLa S3 cell. *Power Modulator and High Voltage Conference (IPMHVC), 2010 IEEE International*.

Mitsutake, K., A. Satoh, et al. (2012). Effect of pulsing sequence of nanosecond pulsed electric fields on viability of HeLa S3 cells. *Dielectrics and Electrical Insulation, IEEE Transactions on* 19(1): 337-342.

Moen, E. K., C. C. Roth, et al. (2013). Changes in protein expression of U937 and Jurkat cells exposed to nanosecond pulsed electric fields. 85850R-85850R.

Movahed, S. and D. Li (2011). Microfluidics cell electroporation. *Microfluidics and Nanofluidics* 10(4): 703-734.

Muramatsu, T., S. Arakawa, et al. (2001). In vivo gene electroporation in skeletal muscle with special reference to the duration of gene expression. *International journal of molecular medicine* 7(1): 37-79.

Nazib Adon, M., M. Noh Dalimin, et al. (2011). Microdosimetry Modeling Technique for Spherical Cell

5th Kuala Lumpur International Conference on Biomedical Engineering 2011. N. A. A. Osman, W. A. B. W. Abas, A. K. A. Wahab and H.-N. Ting, Springer Berlin Heidelberg. 35**:** 447-449.

Neal Ii, R. E., J. H. Rossmeisl Jr, et al. (2014). In vitro and numerical support for combinatorial irreversible electroporation and electrochemotherapy glioma treatment. *Annals of Biomedical Engineering* 42(3): 475-487.

Neu, W. and J. Neu (2009). Theory of Electroporation. *Cardiac Bioelectric Therapy*. I. Efimov, M. Kroll and P. Tchou, Springer US**:** 133-161.

Neuman., E. and K. Rosenheck. (1972). Permeability Cahnges Induced by Electrical Impulses in Vesicular Membranes. *J. Membrane Biol.* 10: 279-290.

Neumann, E. (1989). The relaxation hysteresis of membrane electroporation. *Electroporation and electrofusion in cell biology*, Springer**:** 61-82.

Neumann, E., G. Gerisch, et al. (1980). Cell fusion induced by high electric impulses applied to Dictyostelium. *Naturwissenschaften* 67(8): 414-415.

Neumann, E. and S. Kakorin (2002). Digression on membrane electroporation for drug and gene delivery. *Technology in cancer research & treatment* 1(5): 329-340.

Neumann, E., S. Kakorin, et al. (1999). Membrane electroporation and electromechanical deformation of vesicles and cells. *Faraday Discuss.* 111: 111-125.

Neumann, E. and K. Rosenheck (1972). Permeability changes induced by electric impulses in vesicular membranes. *The Journal of Membrane Biology* 10(1): 279-290.

Neumann, E., M. Schaefer-Ridder, et al. (1982). Gene transfer into mouse lyoma cells by electroporation in high electric fields. *The EMBO journal* 1(7): 841.

Nicolau, C., Y. Mouneimne, et al. (1993). Electroinsertion of proteins in the plasma membrAne of red blood cells. *Analytical Biochemistry* 214(1): 1-10.

Nikolski, V. P. and I. R. Efimov (2005). Electroporation of the heart. *Europace* 7(s2): S146-S154.

O'Neill, R. J. and L. Tung (1991). Cell-attached patch clamp study of the electropermeabilization of amphibian cardiac cells. *Biophysical Journal* 59(5): 1028-1039.

Pakhomova, O. N., B. W. Gregory, et al. (2011). Electroporation-Induced Electrosensitization. *PLoS One* 6(2): e17100.

Pavlin, M., N. Pavselj, et al. (2002). Dependence of induced transmembrane potential on cell density, arrangement, and cell position inside a cell system. *Biomedical Engineering, IEEE Transactions on* 49(6): 605-612.

Pech, M., A. Janitzky, et al. (2011). Irreversible Electroporation of Renal Cell Carcinoma: A First-in-Man Phase I Clinical Study. *CardioVascular and Interventional Radiology* 34(1): 132-138.

Peng, L., D. Obata, et al. (2012). Influence of Intense Pulsed UV Irradiation on the Viability and Proliferation of HeLa Cells. *Plasma Science, IEEE Transactions on* 40(8): 2020-2027.

Petrishia, A. and M. Sasikala (2014). Design of tapered arm impulse radiating antenna with log periodic lens system for skin cancer treatment. *Journal of Medical Engineering and Technology* 38(3): 135-145.

Pohl, H. A. (1978). Dielectrophoresis: The Behavior of Matter in Non-uniform Electric Fields, Cambridge University Press Cambridge.

Potter, H. (1988). Electroporation in biology: methods, applications, and instrumentation. *Analytical Biochemistry* 174(2): 361-373.

Prausnitz, M. R., V. G. Bose, et al. (1993). Electroporation of mammalian skin: A mechanism to enhance transdermal drug delivery. *Proceedings of the National Academy of Sciences of the United States of America* 90(22): 10504-10508.

Prausnitz, M. R., U. Pliquett, et al. (1994). Rapid temporal control of transdermal drug delivery by electroporation. *Pharmaceutical Research* 11(12): 1834-1837.

Raso-Pueyo, J. and V. Heinz (2010). *Pulsed electric fields technology for the food industry: fundamentals and applications*, Springer.

Rebers, x030C, et al. (2014). Cell membrane electroporation-Part 3: the equipment. *Electrical Insulation Magazine, IEEE* 30(3): 8-18.

Reigada, R. and M. L. Fernandez (2011). Structure and electroporation of lipid bilayers: A Molecular Dynamics study. *General Assembly and Scientific Symposium, 2011 XXXth URSI.*

Richmond, J. H. (1965). Digital computer solutions of the rigorous equations for scattering problems. *Proceedings of the IEEE* 53(8): 796-804.

Rosenheck, K., P. Lindner, et al. (1975). Effect of electric fields on light-scattering and fluorescence of chromaffin granules. *The Journal of Membrane Biology* 20(1): 1-12.

Roy, T. and S. Barman (2014). A behavioral study of healthy and cancer genes by modeling electrical network. *Gene* 550(1): 81-92.

Saito, K., Y. Minamitani, et al. (2013). Investigation of selective sterilization of unnecessary microorganisms on pulsed electric field sterilization. *Digest of Technical Papers-IEEE International Pulsed Power Conference*.

Sale, A. and W. Hamilton (1967). Effects of high electric fields on microorganisms: I. Killing of bacteria and yeasts. *Biochimica et Biophysica Acta (BBA)-General Subjects* 148(3): 781-788.

Salipante, P. F. and P. M. Vlahovska (2014). Vesicle deformation in DC electric pulses. *Soft Matter* 10(19): 3386-3393.

Sancho, M., G. Martínez, et al. (2003). Accurate dielectric modelling of shelled particles and cells. *Journal of Electrostatics* 57(2): 143-156.

Sano, M. B., C. B. Arena, et al. In-vitro bipolar nano- and microsecond electro-pulse bursts for irreversible electroporation therapies. *Bioelectrochemistry*(0).

Sano, M. B., C. B. Arena, et al. (2014). In-vitro bipolar nano- and microsecond electro-pulse bursts for irreversible electroporation therapies. *Bioelectrochemistry*.

Satkauskas, S. and G. Saulis (2004). Electroporation as a tool for biotechnology and medicine with specific emphasize on its application for drug and gene delivery. Review. *Veterinarija ir Zootechnika* 26(48): 74.

Saulis, G. (2010). Electroporation of cell membranes: the fundamental effects of pulsed electric fields in food processing. *Food Engineering Reviews* 2(2): 52-73.

Saulis, G. and R. Saulė (2012). Size of the pores created by an electric pulse: Microsecond vs millisecond pulses. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1818(12): 3032-3039.

Saunders, J. A., C. R. Smith, et al. (1989). Effects of Electroporation Pulse Wave on th e Incorporation of Viral RNA into Tobacc o Protoplasts.

Scheurich, P., U. Zimmermann, et al. (1981). Electrically Stimulated Fusion of Different Plant Cell Protoplasts MESOPHYLL CELL AND GUARD CELL PROTOPLASTS OF VICIA FABA. *Plant physiology* 67(4): 849-853.

Schmidt, E., U. Leinfelder, et al. (2001). CD19+ B lymphocytes are the major source of human antibody-secreting hybridomas generated by electrofusion. *Journal of immunological methods* 255(1): 93-102.

Schönenberger, C., A. Schütz, et al. (2011). Efficient electroporation of peptides into adherent cells: investigation of the role of mechano-growth factor in chondrocyte culture. *Biotechnology Letters* 33(5): 883-888.

Schwan, H. (1977). Field interaction with biological matter. *Annals of the New York Academy of Sciences* 303: 198.

Schwan, H. P. (1957). Electrical properties of tissue and cell suspensions. *Advances in biological and medical physics* 5: 147.

Schwan, H. P. (1971). Interaction of microwave and radio frequency radiation with biological systems. *Microwave Theory and Techniques, IEEE Transactions on* 16(2): 146-152.

Schwister, K. and B. Deuticke (1985). Formation and properties of aqueous leaks induced in human erythrocytes by electrical breakdown. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 816(2): 332-348.

Sersa, G., D. Miklavcic, et al. (2008). Electrochemotherapy in treatment of tumours. *European Journal of Surgical Oncology (EJSO)* 34(2): 232-240.

Shillcock, J. C. and U. Seifert (1998). Thermally Induced Proliferation of Pores in a Model Fluid Membrane. *Biophysical Journal* 74(4): 1754-1766.

Silve, A., A. G. Brunet, et al. (2014). Comparison of the effects of the repetition rate between microsecond and nanosecond pulses: Electropermeabilization-induced electro-desensitization? *Biochimica et Biophysica Acta (BBA)-General Subjects* 1840(7): 2139-2151.

Simoes, S., V. Slepushkin, et al. (1998). Gene delivery by negatively charged ternary complexes of DNA, cationic liposomes and transferrin or fusigenic peptides. *Gene Therapy* 5(7): 955-964.

Smith, K. C., J. C. Neu, et al. (2004). Model of Creation and Evolution of Stable Electropores for DNA Delivery. *Biophysical Journal* 86(5): 2813-2826.

Smith, W. D. (1975). The application of finite element analysis to body wave propagation problems. *Geophysical Journal International* 42(2): 747-768.

Snoj, M., Z. Rudolf, et al. (2005). Successful sphincter-saving treatment of anorectal malignant melanoma with electrochemotherapy, local excision and adjuvant brachytherapy. *Anti-Cancer Drugs* 16(3): 345-348.

Son, R., K. Smith, et al. (2014). Basic Features of a Cell Electroporation Model: Illustrative Behavior for Two Very Different Pulses. *The Journal of Membrane Biology*: 1-20.

Tattersall, J. E., S. J. Wood, et al. (1999). The effects of radiofrequency electromagnetic fields on the electrophysiology of rat brain slices in vitro. *Electromagnetic Assessment and Antenna Design Relating To Health Implications of Mobile Phones (Ref. No. 1999/043), IEE Seminar on*, IET.

Teissie, J. and T. Y. Tsong (1981). Electric field induced transient pores in phospholipid bilayer vesicles. *Biochemistry* 20(6): 1548-1554.

Tekle, E., R. D. Astumian, et al. (1991). Electroporation by using bipolar oscillating electric field: an improved method for DNA transfection of NIH 3T3 cells. *Proceedings of the National Academy of Sciences* 88(10): 4230-4234.

Tekle, E., R. D. Astumian, et al. (1994). Selective and asymmetric molecular transport across electroporated cell membranes. *Proceedings of the National Academy of Sciences* 91(24): 11512-11516.

Thompson, G., J. A. Payne, et al. (2011). Local plasma membrane permeabilization of living cells by nanosecond electric pulses using atomic force microscopy.

Thompson, G. L., C. C. Roth, et al. (2014). Calcium influx affects intracellular transport and membrane repair following nanosecond pulsed electric field exposure. *Journal of Biomedical Optics* 19(5).

Titomirov, A. V., S. Sukharev, et al. (1991). In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression* 1088(1): 131-134.

Tobin, A. J. and R. E. Morel (1996). *Asking About Cells*, Saunders College Publishing.

Toepfl, S., V. Heinz, et al. (2007). High intensity pulsed electric fields applied for food preservation. *Chemical engineering and processing: Process intensification* 46(6): 537-546.

Tokmakçı, M. (2006). A High-Voltage Pulse Generation Instrument for Electrochemotherapy Method. *Journal of Medical Systems* 30(3): 145-151.

Tozon, N., V. KODRE, et al. (2005). Effective treatment of perianal tumors in dogs with electrochemotherapy. *Anticancer research* 25(2A): 839-845.

Tsong, T. Y. (1991). Electroporation of cell membranes. *Biophysical Journal* 60(2): 297-306.

Tsuru, M., K. Nagata, et al. (2002). Confocal Laser Microscopy of Chondrocytes That Received Gene Transfer Using in vitro Electroporation. *Kurume Medical Journal* 49(1-2): 1-5.

Ušaj, M., K. Trontelj, et al. (2010). Cell–Cell Electrofusion: Optimization of Electric Field Amplitude and Hypotonic Treatment for Mouse Melanoma (B16-F1) and Chinese Hamster Ovary (CHO) Cells. *The Journal of Membrane Biology* 236(1): 107-116.

Valič, B., M. Golzio, et al. (2003). Effect of electric field induced transmembrane potential on spheroidal cells: theory and experiment. *European Biophysics Journal* 32(6): 519-528.

Valič, B., M. Pavlin, et al. (2004). The effect of resting transmembrane voltage on cell electropermeabilization: a numerical analysis. *Bioelectrochemistry* 63(1): 311-315.

Vernhes, M., A. Benichou, et al. (2002). Elimination of free-living amoebae in fresh water with pulsed electric fields. *Water Research* 36(14): 3429-3438.

Wang, S. M., Y. J. Tsai, et al. (1997). Studies on the function of rho a protein in cardiac myofibrillogenesis. *Journal of Cellular Biochemistry* 66(1): 43-53.

Wang, Z. (2009). Electromagnetic Field Interaction with Biological Tissues and Cells, Queen Mary, University of London.

Weaver, J. C. (1993). Electroporation: a general phenomenon for manipulating cells and tissues. *Journal of Cellular Biochemistry* 51: 426-426.

Weaver, J. C. (2000). Electroporation of cells and tissues. *Plasma Science, IEEE Transactions on* 28(1): 24-33.

Weaver, J. C. (2003). Electroporation of biological membranes from multicellular to nano scales. *Dielectrics and Electrical Insulation, IEEE Transactions on* 10(5): 754-768.

Weaver, J. C. and Y. A. Chizmadzhev (1996). Theory of electroporation: A review. *Bioelectrochemistry and Bioenergetics* 41(2): 135-160.

Weiland, T. (1977). A discretization model for the solution of Maxwell's equations for six-component fields. *Archiv Elektronik und Uebertragungstechnik* 31: 116-120.

Wells, D. J. (2010). Electroporation and ultrasound enhanced non-viral gene delivery in vitro and in vivo. *Cell Biology and Toxicology* 26(1): 21-28.

Wezgowiec, J., M. Kotulska, et al. (2013). Cyanines in photodynamic reaction assisted by reversible electroporation-in vitro study on human breast carcinoma cells. *Photodiagnosis and Photodynamic Therapy* 10(4): 490-502.

Wong, T.-K. and E. Neumann (1982). Electric field mediated gene transfer. *Biochemical and Biophysical Research Communications* 107(2): 584-587.

Yang, H. Q., Y. H. Wang, et al. (2012). Efficacy of Proliferation of HeLa Cells under Three Different Low-Intensity Red Lasers Irradiation. *International Journal of Photoenergy* 2012: 5.

Yang, M., K. F. Ren, et al. (2013). Computation of radiation pressure force on arbitrary shaped homogenous particles by multilevel fast multipole algorithm. *Optics letters* 38(11): 1784-1786.

Yarmush, M. L., A. Golberg, et al. (2014). Electroporation-Based Technologies for Medicine: Principles, Applications, and Challenges. *Annual Review of Biomedical Engineering* 16(1): 295-320.

Yee, K. S. (1966). Numerical solution of initial boundary value problems involving Maxwell's equations in isotropic media. *IEEE Trans. Antennas Propag* 14(3): 302-307.

Yu, J., L. Qiao, et al. (2006). Troglitazone inhibits tumor growth in hepatocellular carcinoma in vitro and in vivo. *Hepatology* 43(1): 134-143.

Zhang, M., Z. A. Xiong, et al. (2013). Intense picosecond pulsed electric fields inhibit proliferation and induce apoptosis of HeLa cells. *Molecular Medicine Reports* 7(6): 1938-1944.

Zhang, X. T., L. G. Kang, et al. (2011). A positive feedback loop of ER-[alpha]36/EGFR promotes malignant growth of ER-negative breast cancer cells. *Oncogene* 30(7): 770-780.

Zimmerman., U., G. Pilwat., et al. (1974). Dielectric Breakdown of Cell Membrane. *Biophys. J* 14: 881-899.

Zimmermann, U. (1982). Electric field-mediated fusion and related electrical phenomena. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes* 694(3): 227-277.

Zimmermann, U., U. Friedrich, et al. (2000). Electromanipulation of mammalian cells: fundamentals and application. *Plasma Science, IEEE Transactions on* 28(1): 72-82.